IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Thomas FELZMANN

Application No.: 10/527,679 | Confirmation No.: 7223

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For: USE OF DENDRITIC CELLS (DCS) EXPRESSING INTERLEUKIN 12 (IL-12) | Examiner: X. Xie

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Thomas Felzmann, a citizen of Austria and residing at Weideckerstraße 19-21/6, 3004, Riederberg, Austria say and declare as follows:

1. I received the degree Medical Doctor from University of Vienna Medical School in the year of 1987.

2. I have worked at the St. Anna Children's Hospital & Cancer Research Institute from 1995 to 2003.

3. I worked at Trimed Biotech GmbH, formerly I-Med Krebsimmuntherapie GmbH, from 2004 to May 2008. I have been studying the biology of the regulation of anti-tumor immunity regulated by dendritic cells and its application to cancer treatment for the last 13 years.

4. I am a member of the Austrian Society for Immunology and Allergology.

5. I am an author or co-author of the papers of the list attached.

First, last or corresponding author manuscripts:

Dohnal AM, Graffi S, Witt V, Eichstill C, Wagner D, Ul-Haq S, Wimmer D, Felzmann T. Comparative evaluation of techniques for the manufacturing of dendiric cell-base cancer vaccines J Cell Mol Med. 2008 Mar 17. [Epub ahead of print]

Dohnal A, Witt V, Hugel H, Holter W, Gadner H, Felzmann T. Phase I study of tumor Ag-loaded IL-12 secreting semi-mature DC for the treatment of pediatric cancer. Cytotherapy. 2007 Oct;9(8):755-70

Hüttner KG, Breuer SK, Paul P, Majdic O, Heitger A, Felzmann T. Generation of potent anti-tumor immunity in mice by interleukin-12-secreting dendritic cells. Cancer Immunol Immunother. 2005 Jan;54(1):67-77.

Felzmann T, Hüttner KG, Breuer SK, Wimmer D, Ressmann G, Wagner D, Paul P, Lehner M, Heitger A, Holter W. Semi-mature IL-12 secreting dendritic cells present exogenous antigen to trigger cytolytic immune responses. Cancer Immunol Immunother. 2005 Aug;54(8):769-80.

Felzmann T, Witt V, Wimmer D, Ressmann G, Wagner D, Paul P, Huttner K, Fritsch G. Monocyte enrichment from leukapharesis products for the generation of DCs by plastic adherence, or by positive or negative selection. Cytotherapy. 2003;5(5):391-8.

Felzmann T, Gadner H, Holter W. Dendritic cells as adjuvants in antitumor immune therapy. Onkologie. 2002 Oct;25(5):456-64.

Felzmann T, Buchberger M, Lehner M, Printz D, Kircheis R, Wagner E, Gadner H, Holter W. Functional maturation of dendritic cells by exposure to CD40L transgenic tumor cells, fibroblasts or keratinocytes. Cancer Lett. 2001 Jul 26;168(2):145-54.

Felzmann T, Buchberger M, Jechlinger M, Kircheis R, Wagner E, Gadner H. Xenogenization by tetanus toxoid loading into lymphoblastoid cell lines and primary human tumor cells mediated by polycations and liposomes. Cancer Lett. 2000 Dec 20;161(2):241-50.

Felzmann T, Ramsey WJ, Blaese RM. Anti-tumor immunity generated by tumor cells engineered to express B7-1 via retroviral or adenoviral gene transfer. Cancer Lett. 1999 Jan 8;135(1):1-10.

Felzmann T, Ramsey WJ, Blaese RM. Characterization of the antitumor immune response generated by treatment of murine tumors with recombinant adenoviruses expressing HSVtk, IL-2, IL-6 or B7-1. Gene Ther. 1997 Dec;4(12):1322-9.

Felzmann T, Gisslinger H, Ludwig H. Immunological findings in patients with myelodysplastic syndrome. Leuk Lymphoma. 1994 Oct;15(3-4):201-8. Review.

Felzmann T, Gisslinger H, Krieger O, Majdic O, Ludwig H, Koller U. Immunophenotypic characterization of myelomonocytic cells in patients with myelodysplastic syndrome. Br J Haematol. 1993 Jul;84(3):428-35.

Felzmann T, Gadd S, Majdic O, Maurer D, Petera P, Smolen J, Knapp W. Analysis of function-associated receptor molecules on peripheral blood and synovial fluid granulocytes from patients with rheumatoid and reactive arthritis. J Clin Immunol. 1991 Jul;11(4):205-12.

Co-author manuscripts:

Lehner M, Stockl J, Majdic O, Knapp W, Huttner K, Felzmann T, Holter W. MHC class II antigen signaling induces homotypic and heterotypic cluster formation of human mature monocyte derived dendritic cells in the absence of cell death. Hum Immunol. 2003 Aug;64(8):762-70.

Lehner M, Felzmann T, Clodi K, Holter W. Type I interferons in combination with bacterial stimuli induce apoptosis of monocyte-derived dendritic cells. Blood. 2001 Aug 1;98(3):736-42.

Kircheis R, Kichler A, Wallner G, Kursa M, Ogris M, Felzmann T, Buchberger M, Wagner E. Coupling of cell-binding ligands to polyethylenimine for targeted gene delivery. Gene Ther. 1997 May;4(5):409-18.

Maurer D, Felzmann T, Holter W, Petera P, Smolen J, Knapp W. Evidence for the presence of activated CD4 T cells with naive phenotype in the peripheral blood of patients with rheumatoid arthritis. Clin Exp Immunol. 1992 Mar;87(3):429-34.

Gadd SJ, Felzmann T, Majdic O, Maurer D, Petera P, Chen WJ, Smolen J, Knapp W. Phenotypic analysis of functionally associated molecules on peripheral blood and synovial fluid monocytes from arthritis patients. Rheumatol Int. 1992;12(4):153-7.

Szekeres T, Fritzer M, Pillwein K, Felzmann T, Chiba P. Cell cycle dependent regulation of IMP dehydrogenase activity and effect of tiazofurin. Life Sci. 1992;51(16):1309-15.

Maurer D, Fischer GF, Felzmann T, Majdic O, Gschwantler E, Hinterberger W, Wagner A, Knapp W. Ratio of complement receptor over Fc-receptor III expression: a sensitive parameter to monitor granulocyte-macrophage colony-stimulating factor effects on neutrophils. Ann Hematol. 1991 Apr;62(4):135-40.

Maurer D, Felzmann T, Knapp W. A single laser flow cytometry method to evaluate the binding of three antibodies. J Immunol Methods. 1990 Dec 31;135(1-2):43-7.

6. I am one of the inventors in U.S. Patent Application Serial Number 10/527,679. I am very familiar with the subject matter thereof and have been researching the subject matter thereof since 1995.

7. I have performed, or supervised the performance of, the experiments described in the following paragraphs in support of patentability of the above-identified patent application.

8. Dendritic cells (DC) are the master switches of immunity. They have the capacity to flexibly respond to pathogen associated molecular patterns (PAMP) and/or danger signals guiding a developing immune response accordingly. Tumors develop various mechanisms to escape immune surveillance, many of which result in a suppression of the function of DCs. A common goal in anti-tumor immune therapeutic strategies is, hence, to conduct immune-activation against tumor antigens under controlled in vitro conditions.

9. DCs may be found in every tissue of an organism. Their default function is to maintain tolerance against auto-antigens thus preventing the immune system from developing an autoimmune disease. Upon encountering a PAMP/danger signal, they start to differentiate/mature into an immune stimulatory phenotype. Depending on the nature of the danger signal or PAMP the resulting immune response is polarized towards killer cell activity, or towards antibody production. This polarization is guided by the release of cytokines from the DC, most critically IL-12. Is an antigen presented from the DC together with the delivery of co-stimulatory signals to a T-cell in the presence of IL-12, the immune response is polarized to favor killer cell activity. In order to initiate a killer cell mediated anti-tumor immune response - antibodies typically do not have the capacity to kill target cells but rather serve as a "red flag" recruiting other immune cells - it is therefore critical that IL-12 is secreted from the DC during tumor antigen presentation.

10. However, IL-12 secretion is limited to a narrow time window of 24 hours following encounter of a danger signal or PAMP. After that, the DC goes on to assume yet another functional phenotype which is mainly active in down-modulating immunity.

These immune suppressive feedback loops are initiated by the same molecular encounter of a danger signal or PAMP that started the functional differentiation of the DC but become dominate with a time delay of 24-48 hours in order to prevent an immune response from running out of control.

LPS represents bacterial endotoxin and is the substance responsible for lethal endotoxin shocks during a bacterial sepsis. Therefore, the use of LPS as a PAMP to initiate DC differentiation/maturation was considered impossible because of the related potential for adverse events. Only our later experiments indicated that it is feasible after all to use bacterial endotoxin as a PAMP to deliver a differentiation/maturation signal to DCs. An extended experimental series was necessary to work out the exact conditions under which LPS and IFN-γ may be used in a clinical setting without causing harm to the patient.

11. Felzmann et al. (2001) was focused on the function of the T-cell derived CD40L signal that is received by the DC via its CD40 molecule which also has the capacity to initiate the DC differentiation/maturation process. This earlier study was designed exclusively to understand the signaling involved in the initiation of the DC differentiation/maturation process. Although LPS was used to deliver a PAMP to the DCs in the presence of IFN-γ, at the time there was no intention to further develop such a system into a cancer treatment. This is also suggested by the choice of test antigen used in these experiments.

Epstein-Barr virus (EBV) is a very common virus. Up to 90% of humans have encountered EBV and developed strong recall immunity, typically during their childhood. This memory immunity protects them similar to a vaccine from the development of disease at a secondary contact with EBV. Although the first contact with EBV is typically accompanied by only mild flu like symptoms, people infected with EBV for the first time may develop the symptoms of "Infectious Mononucleosis". Only in the rare case of immune compromised patients will an EBV infection have the capacity to develop into a Burkit Lymphoma. Tumor antigens are in fact auto-antigens. The immune system is

actively adapted and tolerant against the tumor antigens. This means that in order to trigger a killer cell mediated immune response directed against tumor antigens, the tolerance of the immune system against tumor antigens needs to be broken. This equals the development of an autoimmune disease.

Many safeguards protect an organism from an autoimmune disease, which all need to be circumvented when a treatment strategy aims at enlisting a cancer patients own immune system. In contrast, the induction of an immune response against viral antigens particularly when memory immunity is already established is much easier in any experimental system as well as in a patient. Thus, using EBV derived antigens presented from DCs to T-cells and measuring the resulting T-cell stimulation does not allow any conclusions concerning the capacity of such dendritic cells to trigger anti-tumor autoimmunity. The experiments described in Felzmann et al. (2001) are therefore not suitable to establish the fact that DCs may be used in the context of a cancer vaccine. Furthermore, knowing that this experimental setting is not suitable for measuring anti-tumor immunity, no killer cell assays were performed. Only the proliferation of T-cells upon exposure to EBV loaded DCs was measured.

In Felzmann et al. (2001) the DCs were not loaded with tumor antigen so it was essentially impossible to draw any conclusions about the development of a cytolytic anti-tumor autoimmune response from that data. In fact, no analysis of killer cell function was done at all. The focus of those investigations was on the DC alone.

12. Most critically, as outlined above, the initiation of an immune response based on killer cells is only possible during the 24 hours time window in which IL-12 is secreted from the DCs. No other investigators had considered the need to initiate the interaction of DCs and T-cells early after exposure of the DCs to the PAMP/danger signal in the presence of IL-12. There is a very critical reason for that, which makes the present invention not obvious: co-stimulation is an absolute critical factor in DC dependent T-cell activation. Exposure to a PAMP/danger signal triggers the up-regulation of co-stimulatory membrane molecules. However, the expression density on the DC surface

reaches its maximum only after 48 hours. Thus, the dogma in immunology was, and still is, that the DC/T-cell interaction needs to be started 48 hours after delivery of the PAMP/danger signal to the DC when the expression density of co-stimulatory molecules reaches its maximum. Of course, at that time IL-12 secretion has completely ceased and it is impossible to prime killer cells against tumor antigens.

13. What was learned and what became a critical feature of the present invention is the fact that a short exposure of DCs to a PAMP/danger signal is sufficient to initiate the differentiation program of DCs, even when the stimulatory molecules are removed from the system. This is of course necessary in the case of LPS before inoculation into the patient during the 24 hours time window in which DCs secrete IL-12 and are capable of killer cell priming. If DCs are inoculated as a medicament into a patient only after 48 hours, the capacity for killer cell activation in the patient is gone. In fact, it might even be dangerous for the patient because of the above mentioned immune suppressive feedback loops that become active in the DCs between 24-48 hours and have the capacity to suppress anti-tumor immunity rather than to initiate it.

14. The undersigned declares further that all statement made herein of his own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of above identified application or any patent issuing thereon.

Date:

Vienna, 13.05.08

Signature:

T. Feltmann

[name of declarant]

[THOMAS FELTMANN]